AMENDMENTS

In The Specification:

Please amend the specification by replacing the specified paragraphs with those shown below. The changes to each paragraph are detailed in the attached Appendix.

Page 1, lines 12 to 19:

The utility of the present invention is to develop a vaccine against the intracellular pathogens, which are causative agents of tuberculosis, brucellosis, leishmaniasis, listeriosis, leprosy, malaria, typhoid, trypanosomiasis and streptococcus and HIV-infection. The pathogen *Mycobacterium tuberculosis* (*M. tuberculosis*) the subject matter of this invention is a causative agent of tuberculosis. In this invention *M. tuberculosis* was allowed to grow in the allogeneic and syngeneic macrophages and macrophage cell lines. The macrophages-*M. tuberculosis* complex was then irradiated to kill the macrophages as well as the mycobacterium.

Page 2, line 23 to page 3, line 3:

A new question has arisen regarding the safety of BCG in HIV-infected individuals. A small number of cases of disseminated BCG-osis have been reported among children who received BCG vaccine and were subsequently found to be HIV seropositive (Von Reyn, et. al. *Lancet* 1987: ii:669-672; Braun, et. al., *Pediatr. Infect. Dis. J.* 1992:11:220-227; Weltman, et. al., *AIDS* 7:1993:149). WHO currently recommends discontinuing the use of BCG vaccine in children showing overt signs of immunodeficiency (World Health Organization, 1992, *Expanded Program for Immunization, Program Report*,World Health Organization, Geneva; *Weekly Epidemiol. Rec.* 62:1987:53).

Page 6, lines 8 to 18:

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The main rationale behind this process was to develop a vaccine against tuberculosis and other intracellular diseases, MHC-matched (syngeneic) and mismatched (allogeneic) macrophages harboring *M. tuberculosis* on irradiation undergo apoptosis; dendritic cells engulf these macrophages and present the antigen (Mycobacterium-proteins and allo-macrophage peptides) on their surface and induce naïve T-cells to differentiate into effecter $CD4^+$ Th1 cells. These dendritic cells also activate $CD8^+$ T cells for cell-mediated immunity. Allo-macrophages in the system generate an allo-reaction and as a result a large amount of cytokines like IL-2, IL-12, IFN-γ, etc., are produced which promote the Th1 response and cell mediated immune response. It is known that Th1-type of response provides protection against tuberculosis. Hence the main utility of the process was to produce a potent and specific vaccine against *M. tuberculosis*.

Page 6, lines 21 to 24:

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The main object of the present invention thus is to develop a vaccine against tuberculosis and other intracellular diseases like leprosy, leishmaniasis, typhoid, trypanosomiasis, malaria, brucellosis, listeriosis, AIDS, streptococcal infection and cancer.

Page 7, lines 5 to 10:

A5

Another object is to develop a vaccine that acts against both syngeneic macrophages entrapped pathogens (viz. M. tuberculosis, M. leprae, leishmania, salmonella, trypanosoma, malaria, brucella, listeria, HIV, streptococcus) (e.g. SMTV, S=syngeneic, M=macrophage, T=tuberculosis, V=vaccine) and allogeneic-macrophages entrapped

pathogen vaccine (e.g., AMTV, A=allo, M=macrophage, T=tuberculosis, V=vaccine), to generate protective immune response.

Page 8, lines 1 to 5:

The vaccine was used after irradiation and the irradiated cells are known to undergo apoptosis. The cells undergoing apoptosis were engulfed by the dendritic cells. Dendritic cells activated naïve T cells to differentiate into Th1 cells and cytotoxic cells. These cells are known to be cardinal in imparting protective immunity against intracellular infections and cancer.

Page 13, line 12 to page 14, line 5:

Accordingly, the present invention provides a vaccine against tuberculosis and other intracellular pathogens selected from the group consisting of *Mycobacterium leprae, leishmania, salmonella, trypanosoma, plasmodium, brucella, listeria, HIV, streptococcus* and *cancer*. The invention also provides a method for the development of the said vaccine, comprising the steps of:

(i) culturing pathogens selected from the group comprising *Mycobacterium tuberculosis*, *Mycobacterium leprae, leishmania, salmonella, trypanosoma, plasmodium, brucella, listeria, HIV,* and *streptococcus*;

(ii) culturing syngeneic (same strain), allogeneic (different strain) and xenogeneic (different species like sheep and goat) macrophages and macrophage cell lines selected from the group consisting of J774, P388D1, RAW, BMC-2, THP-1, etc.;

(iii) infecting macrophages and cell lines with a pathogen;

(iv) treating the infected cells with known drugs followed by gamma irradiation to obtain the vaccine;

(v) immunizing disease resistant and susceptible strains of animals with the vaccine obtained above;

(vi) infecting the animals with live pathogen and monitoring their mortality and viable counts of infectious agent in lungs, spleen and liver; and

(vii) monitoring the vaccinated animals for proliferation and generation of $CD4^+$ Th1 and Th2 cells and $CD8^+$ cytotoxic T cells indicating the generation of cell mediated immunity.

Page 14, lines 6 to 26:

The invention further provides a process for the preparation of a vaccine against tuberculosis, wherein the said process comprising the steps of:

(i) culturing of *Mycobacterium tuberculosis* H37Rv;

(ii) culturing of syngeneic and allogeneic macrophages and macrophage cell lines selected from the group consisting of J774, P388D1, RAW, BMC-2, THP-1, etc.;

(iii) infecting macrophages and cell lines (J774, P388D1, RAW, BMC-2, THP-1) with *M. tuberculosis*;

(iv) treating the infected cells with isoniazid and gamma irradiation to obtain the vaccine;

(v) immunizing tuberculosis resistant and susceptible strains of mice with allogeneic macrophage

tuberculosis vaccine (AMTV) and syngeneic macrophage tuberculosis vaccine (SMTV) obtained above;

(vi) infecting the mice with live *M. tuberculosis* and monitoring their mortality and viable counts of bacteria in lungs, spleen and liver;

(vii) monitoring the vaccinated animals for proliferation and generation of $CD4^+$ Th1 and Th2 cells and $CD8^+$ cytotoxic T cells indicating the generation of cell mediated immunity; and

(viii) inoculating the vaccine in the mouse footpad and examining the delayed type hypersensitivity reaction by measuring the swelling in the footpad for protective immunity.

Page 14, line 27 to page 15, line 18:

The invention also provides a process for the preparation of a vaccine against salmonella, wherein the said process comprising the steps of:

(i) culturing of *Salmonella typhimurium*;

(ii) culturing of syngeneic and allogeneic macrophages and macrophage cell lines selected from the group consisting of J774, P388D1, RAW, BMC-2, THP-1, etc.;

(iii) infecting macrophages and cell lines (J774, P388D1, RAW, BMC-2, THP-1) with *S. typhimurium*;

(iv) treating the infected cells with mitomycin C and gamma irradiation to obtain the vaccine;

(v) immunizing tuberculosis resistant and susceptible strains of mice with the vaccine obtained above;

(vi) infecting the mice with live *S. typhimurium* and monitoring their mortality and viable counts of bacteria in lungs, spleen and liver;

(vii) monitoring the vaccinated animals for proliferation and generation of $CD4^+$ Th1 and Th2 cells and $CD8^+$ cytotoxic T cells indicating the generation of cell mediated immunity; and

(viii) inoculating the vaccine in the mouse footpad and examining the delayed type hypersensitivity reaction by measuring the swelling in the footpad for protective immunity.

Page 15, line 27 to page 16, line 3:

The intracellular pathogens viz. Mycobacterium tuberculosis, Mycobacterium leprae, leishmania, salmonella, trypanosoma, plasmodium, brucella, listeria, HIV, streptococcus were cultured in the macrophages of syngeneic and allogeneic mice, macrophages cell lines J774, P338D1, RAW, BMC-2, THP-1 (ATCC, Rockville). The infected cells were treated with isoniazid (20 µg/ml) for 48h at 37 °C/5% CO_2 and irradiated at 0.05 kGy.

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In The Claims:

Please amend claims 1-3 and 5 to read as shown below. The changes to claims 1-3 and 5 are detailed in the attached Appendix.